# Sucrose-phosphate synthase activity in mature rice leaves following changes in growth $CO_2$ is unrelated to sucrose pool size

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## Summary

• Photosynthetic acclimation of C3 plants to elevated atmospheric [$CO_2$] is often attributed to soluble carbohydrate accumulation. We report the effects of modifying the carbohydrate source–sink balance on carbohydrate metabolism in mature leaves and partitioning in vegetative tissues of rice (*Oryza sativa*).

• Plants were grown under ambient atmospheric [$CO_2$] in outdoor, sunlit, environment-controlled chambers. During late vegetative development treatments were changed to high or low [$CO_2$].

• Within 1 d of changing to low [$CO_2$], sucrose-phosphate synthase (SPS) activation was significantly reduced in mature leaves, while soluble invertase activity decreased. Plants switched to high [$CO_2$] showed increases in SPS substrate-saturated and substrate-limited activities and a decline in invertase activity. The changes in SPS activity did not correlate with leaf sucrose pool size. By 9 d after the change from ambient to high [$CO_2$], nonstructural carbohydrates in stems and leaf sheaths increased significantly; > 70% of this increase was due to sucrose accumulation, indicating that excess assimilate was being rapidly exported to vegetative sinks.

• Results indicate that immediately following source–sink modification, regulatory adjustments in key enzymes controlling carbohydrate metabolism were linked to feedforward, rather than feedback, processes.

**Key words:** acclimation, carbon dioxide, photosynthesis, rice, sucrose-phosphate synthase.



## Introduction

Physiological acclimation involves the adjustment of resources in favor of the most limiting process to optimize survival, reproduction and growth under the prevailing environmental conditions (Acock & Pasternak, 1986). Under the present levels of atmospheric $CO_2$, photosynthesis of C3 plants is limited by the oxygenase activity of Rubisco (Bowes, 1991). Although increased atmospheric [$CO_2$] relieves this limitation, long-term exposure can lead to a reduction of Rubisco activity and protein content (Bowes, 1993). As a consequence, photosynthetic capacity is reduced, but net photosynthesis is still usually higher than that at ambient [$CO_2$].

It has been argued that photosynthetic acclimation functions as a means to increase resource use efficiency (Drake *et al.*, 1997). As postulated, acclimation to elevated $CO_2$ may result from the diversion of nitrogen and other resources away from Rubisco synthesis and maintenance to be used in more limiting processes (e.g. sink development). Rice, which is one of the most agronomically important species in the world, shows a pronounced acclimation to long-term exposure of elevated $CO_2$ (Rowland-Bamford *et al.*, 1991) and provides a good model for studying the processes involved. Despite acclimation to high $CO_2$, rice canopy photosynthesis, growth and yield increase in response to increased [$CO_2$] (Baker *et al.*, 1992). Therefore, carbohydrate utilization and transport are

likely to be upregulated to accommodate excess assimilate resulting from increased photosynthetic rates. Rice accumulates sucrose in response to high photosynthetic rates (Rowland-Bamford *et al.*, 1990) and Hussain *et al.* (1999) showed that sucrose-phosphate synthase (SPS) in rice leaves increases in response to long-term growth under elevated [$CO_2$].

It has long been recognized that carbohydrate accumulation in leaves is often associated with an inhibition of photosynthesis. Photosynthetic acclimation to elevated [$CO_2$] is often attributed to carbohydrate feedback effects, which are thought to be linked to the sensing of increased soluble sugars resulting from carbohydrate sink saturation (Arp, 1991; Bowes, 1993). However, the degree of feedback inhibition of photosynthesis and the form in which excess carbohydrate is stored may differ considerably among species (Goldschmidt & Huber, 1992).

Soluble sugars are important in controlling metabolic functions affecting the partitioning of carbohydrates and have been shown to play a role in regulating the expression of photosynthesis genes (Koch, 1996; Smeekens & Rook, 1997; Moore *et al.*, 1999). Sucrose and hexoses have been linked to changes in Rubisco enzyme expression (Krapp *et al.*, 1993), possibly through their metabolism (Moore *et al.*, 1998) and hence may be involved in acclimation to changes in growth [$CO_2$].

Previously, we showed that switching growth [$CO_2$] resulted in rapid changes in expression of the small subunit gene of Rubisco in rice leaves, but that early in the signal transduction pathway it was not correlated with soluble carbohydrate pool sizes (Gesch *et al.*, 1998). In the present study, rice plants grown in ambient $CO_2$ (350 µl $l^{-1}$) were switched to high (700 µl $l^{-1}$) and low (175 µl $l^{-1}$) $CO_2$ late in vegetative growth phase. The objective was to determine the effect of nonintrusively modifying the carbohydrate source–sink balance on carbohydrate metabolism in mature leaves and the partitioning of carbohydrates between source and sink tissues.

## Materials and Methods

### Growth conditions

Rice (*Oryza sativa* L., cv. Lemont) was grown in three sunlit environment-controlled chambers located outdoors in Gainesville, FL, USA. Pickering *et al.* (1994) gave details of chamber design and methods used for controlling environmental set points. The vats for growing the plants were 2.0 × 1.0 m in cross-section and 0.6 m deep, filled with soil to 0.5 m, thus allowing a large rooting volume for paddy-cultured rice. On October 3, 1996, pregerminated seed was sown in 1.0-m rows running north–south and spaced 0.17 m apart. Nine days after sowing, the rice was thinned to a stand density of 250 plants per $m^2$ and a 0.05-m flood was applied and maintained for the rest of the experiment. Before sowing, 8.4 g $m^{-2}$ P and 13.5 g $m^{-2}$ K were incorporated into the top 0.1 m of soil. Nitrogen was applied to the soil as urea at 12.6, 6.3, and 6.3 g $m^{-2}$ at 8, 18, and 25 d after sowing. Black shade-cloth was hung around the perimeter of each chamber and kept at the same height as the plants to reduce border effects.

Rice in all three chambers was grown at a daytime atmospheric growth [$CO_2$] of 350 µl $l^{-1}$ (referred to as ambient $CO_2$). The day : night dry bulb and dewpoint temperatures were maintained at 28 : 21°C and 18 : 14°C, respectively. At 34 d after sowing, the growth [$CO_2$] of one chamber was switched to 175 µl $l^{-1}$ $CO_2$, another was switched to 700 µl $l^{-1}$, while a third chamber was maintained at 350 µl $l^{-1}$. Environmental conditions as well as canopy carbon exchange rate were automatically sampled every 2 s, and 300-s averages were computed and recorded. Environment control conditions were printed and checked daily to ensure identical performance of the chambers during the study. Variability around the desired set points was ±0.25°C for dry bulb temperature, ±0.5 for dewpoint temperature, and ±5 µl $l^{-1}$ for [$CO_2$].

### Leaf and canopy gas exchange measurements

Photosynthesis was measured at the treatment growth [$CO_2$] on the attached uppermost fully expanded leaf, which was the seventh leaf on the main culm, with a Li-Cor LI-6200 Portable Photosynthesis System (Lincoln, NE, USA) equipped with a 0.25-l cuvette. Measurements were made on leaves of three randomly chosen plants on days 1, 3 and 10 of the experiment between 11 : 30 hours and 12 : 30 hours eastern standard time (EST) when the solar photosynthetic photon flux density (PPFD) was saturating at 1200–1500 mmol $m^{-2}$ $s^{-1}$.

Each chamber [$CO_2$] was monitored with a dedicated IR gas analyser (Ultramat 21P, Siemens, Haguenan, France[1]). The gas analysers were calibrated and checked for linearity before and after the experiment with a range of standard [$CO_2$] in $N_2$. Calibration was checked daily with a span gas. Canopy net photosynthesis rate (Pn) was measured as described by Baker *et al.* (1997). Mean Pn values were computed for the day before the switch (day 1) and 9 d after the switch (day 11) using 300-s averages recorded between 11 : 30 hours and 12 : 30 hours EST. These measurements were taken before leaf sampling for biochemical analysis, and while solar PPFD was > 1200 µmol $m^{-2}$ $s^{-1}$. Nitrous oxide was injected into each chamber twice during the day and its draw-down monitored with a separate IR gas analyser (CID, Vancouver, WA, USA) to calculate chamber leakage rate and correct Pn (Acock & Acock, 1989).

### Leaf and plant sampling

For biochemical analysis, the uppermost fully expanded sunlit leaf (leaf seven) was detached at the ligule from 10 to 20 plants

[1] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

between 13 : 00 hours and 15 : 00 hours EST at days 1, 3 and 10 of the experiment. After detachment, the leaves were immediately plunged into liquid $N_2$. Leaves were then pooled by treatment and ground to a fine powder and stored in liquid $N_2$ until analysed. During each sampling, a subset of six leaves was taken to determine the ratio of fresh weight to leaf area. At the beginning of the experiment the eighth leaf on the main culm was emerging and approximately 25% expanded. By the end of the experiment the eighth leaf of ambient $CO_2$ control plants was fully expanded and a ninth leaf was near full expansion.

For whole-plant analysis, nine plants were sampled from each chamber at days 1 and 11. For each sampling a subset of three plants was taken from each one-third of the chamber area. Leaf area was measured with a Model LI-3100 leaf area meter (Li-Cor) and the plant material was oven dried for 48 h at 65°C before measuring final dry weights. Dried material was pooled by tissue type (leaf blade or stem and sheath), ground to a fine powder in a ball mill and then stored in airtight plastic containers until analysed for nonstructural carbohydrates.

## SPS, invertase, and adenosine 5′-diphosphoglucose pyrophosphorylase assays

Sucrose-phosphate synthase was assayed as described by Huber *et al.* (1989), with some modifications. Approximately 150 mg of liquid $N_2$ frozen leaf powder was ground in a precooled Ten Broek homogenizer at 4°C in 2.5 ml of 50 mM 3-[N-Morpholino propanesulfonic acid (MOPS)-NaOH, 15 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid (EDTA), 2.5 mM dithiothreitol (DTT), 0.1% (v/v) Triton X-100, 2% (w/v) Polyvinyl Pyrrolidone (PVP)-40 and 1 mM phenazine methosulfate (PMSF) at pH 7.5. The extract was centrifuged at 12 000 ***g*** for 1 min at 4°C. The supernatant was rapidly desalted on a Sephadex G-25 column equilibrated with extraction buffer. Sucrose-phosphate synthase activity was measured under saturating ($V_{max}$) and limiting ($V_{lim}$) substrate conditions as fructose-6-P-dependent formation of sucrose and sucrose-P from UDP-glucose (Huber *et al.*, 1989). For the $V_{max}$ assay, 45 µl of desalted extract was added to 50 mM MOPS-NaOH, 15 mM $MgCl_2$, 2.5 mM DTT, 10 mM UDP-glucose, 10 mM fructose-6-P, and 40 mM glucose-6-P at pH 7.5 in a total volume of 70 µl. The $V_{lim}$ assay consisted of the same mixture except that 10 mM Pi was added and the concentrations of UDP-glucose, fructose-6-P, and glucose-6-P were reduced to 2, 2 and 10 mM, respectively. The reactions were terminated after 10 min at 30°C with 70 µl of 1 N NaOH and boiled for 10 min to degrade unreacted fructose-6-P. After cooling, 0.25 ml of 0.1% (w : v) resorcinol in 95% ethanol and 0.75 ml of 30% (v : v) HCl were added and the tubes were incubated at 80°C for 8 min After cooling for 5 min, the absorbance was read at 520 nm. Blanks were run in parallel using the complete assay reaction mix with enzyme denatured by boiling. Two separate extractions of leaf powder were each assayed three times.

Soluble acid invertase was assayed at 37°C in a 200-µl reaction volume containing 100 mM citrate phosphate (pH 5.0) and 50 mM sucrose (Huber, 1989). Approximately 150 mg of liquid $N_2$ frozen leaf powder was extracted and desalted using the same procedure as for SPS, with the exception that the extraction buffer was pH 7.0. The reaction was initiated by adding 40 µl of desalted extract. The reactions were terminated at 0 min and 15 min by boiling, and aliquots were removed for analysis of glucose plus fructose using the microtiter method described by Tarpley *et al.* (1993) and reported as sucrose equivalents. Two separate extractions of leaf powder were each assayed three times.

Adenosine 5′-diphosphoglucose pyrophosphorylase (ADP-glucose-PPase) was measured using a two-stage assay, as described by Nakamura *et al.* (1989). Approximately 200 mg of liquid $N_2$ frozen leaf powder was extracted at 4°C in 3 ml of 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-NaOH, 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM DTT and 10% (v/v) glycerol at pH 7.4. The homogenate was centrifuged at 12 000 ***g*** for 1 min at 4°C and the supernatant immediately assayed for ADP-glucose-PPase activity. The assay mix contained 100 mM HEPES-NaOH, 5 mM $MgCl_2$, 4 mM DTT, 3 mM 3-phosphoglycerate, 3 mM disodium pyrophosphate and 2 mM ADP-glucose in a total volume of 0.25 mM at pH 7.4. Assays were initiated by adding 20 µM of extract and incubated for 10 min at 30°C. Reactions were terminated by boiling for 1 min. Samples were diluted with 0.35 ml of deionized water and centrifuged at 12 000 ***g*** for 5 min. A 0.5-ml aliquot of the supernatant was mixed with 15 µl of 10 mM NADP, and the initial absorbance at 340 nm was recorded. Phosphoglucomutase and glucose-6-P dehydrogenase (1 unit each) were added to initiate the second-stage reaction, and maximum increase in absorbance at 340 nm recorded. Activity is reported in terms of the rate of glucose-1-P production. Frozen leaf powder was extracted once and assayed three times.

## Carbohydrate analysis

Approximately 150 mg of liquid $N_2$ frozen leaf powder or 100 mg of dried plant material was extracted three times in 4 ml of 80% (v/v) ethanol at 85°C. Extracts were combined for each sample and brought to a volume of 15 ml, treated with activated charcoal overnight, and glucose, fructose and sucrose were measured using a microtiter procedure described by Tarpley *et al.* (1993). Two to three separate extractions of frozen leaf powder and dried plant material were each assayed three times.

The pellet remaining after the hot ethanol extraction was oven dried overnight at 60°C. Starch was digested from the pellet by adding 1 ml of 0.2 N KOH and incubating in boiling water for 30 min (Rufty & Huber, 1983). After cooling, 0.2 ml of 1 N acetic acid was added and the solution was incubated with 2 ml of acetate buffer (pH 4.6) containing amyloglucosidase (6 units, Boehringer Mannheim, Germany) at 55°C for

1 h. The reaction was terminated in boiling water. After centrifuging at 3500 *g* for 1 min, the resulting supernatant was assayed for glucose (Tarpley *et al.*, 1993). Total nonstructural carbohydrate was calculated by combining glucose, fructose, sucrose, and starch measurements and reported as glucose equivalents.

### Statistical methods

A repeated measures analysis of variance was used to model the data according to a split-plot-in-time approach using the Mixed Repeated Measures Analysis procedure (SAS Institute, Cary, NC, USA) to obtain estimates of the model. The factors used for the model were [$CO_2$] treatment, day of experiment, and [$CO_2$] treatment × day of experiment. A *t*-test was used to compare least square means for significance.

## Results

Sucrose metabolism in rice leaves changed markedly soon after switching growth [$CO_2$]. Both $V_{max}$ and $V_{lim}$ SPS activities (Fig. 1a,b) significantly increased ($P = 0.05$) in mature leaves within 1 d after switching to high $CO_2$, compared with plants maintained at 350 µl $l^{-1}$. By contrast, switching to low $CO_2$ resulted in a significant decline ($P = 0.05$) in SPS $V_{lim}$ activity but no change in $V_{max}$. By day 10, $V_{max}$ activity was similar across treatments, while $V_{lim}$ activity remained greater in leaves switched to high $CO_2$. Activation of SPS (i.e. the ratio of $V_{lim}$ to $V_{max}$) decreased in mature leaves of rice within 24 h of switching to low $CO_2$, while that of high $CO_2$-switched plants was similar to ambient $CO_2$ controls (Fig. 1c). By day 10, activation was significantly greater ($P = 0.05$) in high $CO_2$-switched leaves, whereas it remained less than ambient controls in those switched to low $CO_2$ but was not found to be significantly different.

Soluble acid invertase activity declined significantly ($P = 0.05$) in mature leaves of both high and low $CO_2$-switched plants within 1 d following the switch (Fig. 2a). Those switched to low $CO_2$ showed 43% less activity than ambient $CO_2$ plants at day 3. However, by day 10 there was no difference in soluble invertase activity across treatments.

ADP-glucose-PPase is a key regulatory enzyme in starch synthesis. Switching to high $CO_2$ resulted in a significant ($P = 0.05$) but small (6%) increase in ADP-glucose-PPase activity in leaves at day 3, and by day 10 there was no difference compared with ambient $CO_2$ plants (Fig. 2b). Leaves of plants switched to low $CO_2$ consistently showed about 14% lower ADP-glucose-PPase activity than ambient $CO_2$ plants.

Within 1 d of the switch, hexoses decreased nearly fourfold in leaves of low $CO_2$-switched plants compared with ambient controls, while they did not change in those switched to high $CO_2$ (Fig. 3a), and the trend persisted at day 10. Sucrose decreased in leaves of both high and low $CO_2$-switched plants by day 3 (Fig. 3b). By day 10 sucrose content was similar between

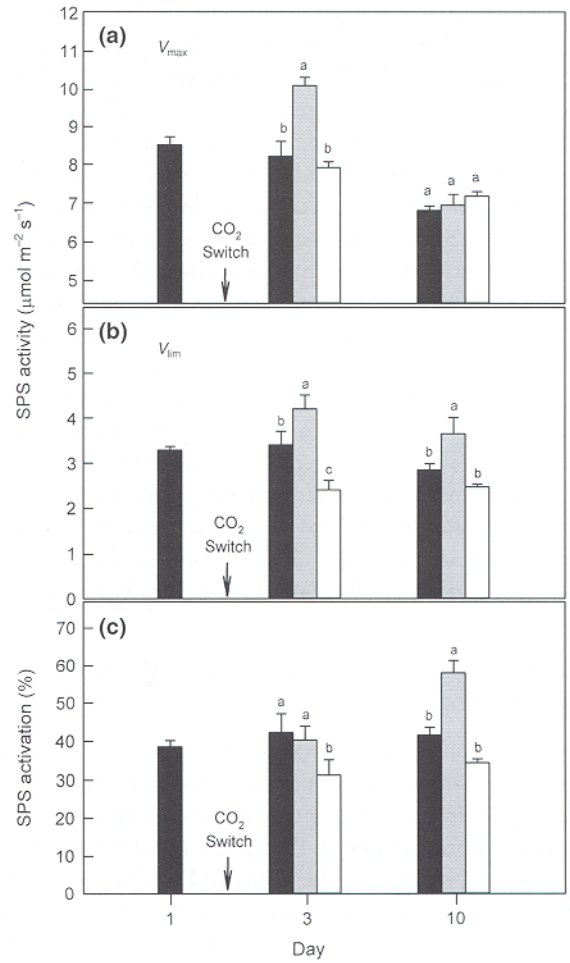


**Fig. 1** Effect of changing growth [$CO_2$] on the enzyme activities of sucrose-phosphate synthase (SPS), $V_{max}$ (a), $V_{lim}$ (b) and percent SPS activation (c) at days 1, 3 and 10 for rice switched to 700 µl $l^{-1}$ and 175 µl $l^{-1}$ $CO_2$ and plants kept at ambient $CO_2$. The $CO_2$ switch was made on day 2. Closed columns, 350 µl $l^{-1}$ $CO_2$; gray columns, 350–750 µl $l^{-1}$ $CO_2$; open columns, 350–175 µl $l^{-1}$ $CO_2$. Values are mean ± SE, $n = 6$. For each day, values followed by a different letter are significantly different at the $P = 0.05$.

high-switched and ambient $CO_2$ leaves but remained significantly less in low $CO_2$-switched plants. Starch was markedly reduced in leaves switched to low $CO_2$, while it was greater in those switched to high $CO_2$ compared with ambient $CO_2$ plants (Fig. 3c). During the experiment both sucrose and starch tended to decline in mature leaves of plants kept at ambient $CO_2$. High $CO_2$-switched leaves also showed a decline in sucrose, but starch increased slightly. Across all $CO_2$ treatments, sucrose content in mature leaves was several-fold higher than starch.

Following the change in growth [$CO_2$], leaf photosynthesis increased by 42% in high $CO_2$-switched plants and decreased by 48% in those switched to low $CO_2$ compared with ambient controls (Fig. 4). By day 10 leaf photosynthesis remained significantly ($P = 0.05$) greater and lower in plants switched to high and low $CO_2$, respectively, compared with ambient

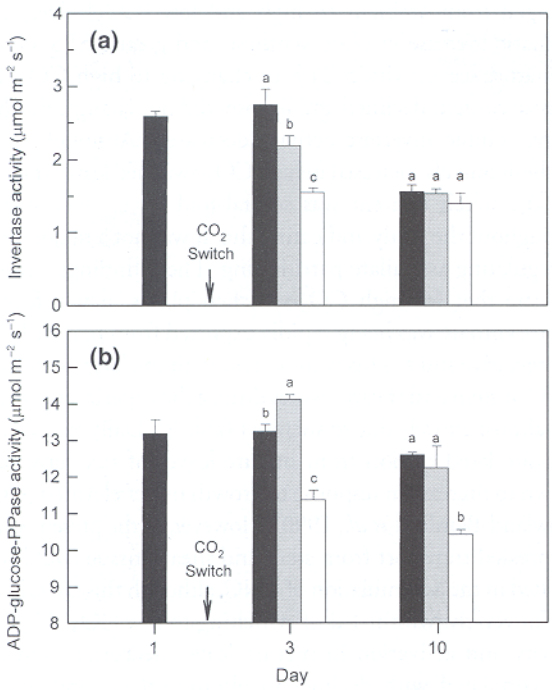


**Fig. 2** Effect of growth [$CO_2$] change on acid soluble invertase (a) and ADP-glucose pyrophosphorylase (b) activity at days 1, 3 and 10 for rice switched to 700 μl $l^{-1}$ and 175 μl $l^{-1}$ $CO_2$ and plants kept at ambient $CO_2$. The $CO_2$ switch was made on day 2. Closed columns, 350 μl $l^{-1}$ $CO_2$; gray columns, 350–750 μl $l^{-1}$ $CO_2$; open columns, 350–175 μl $l^{-1}$ $CO_2$. Values for (a) are mean ± SE, $n$ = 6, and values for (b) are mean ± SD, $n$ = 3. For each day, values followed by a different letter are significantly different at the $P$ = 0.05.

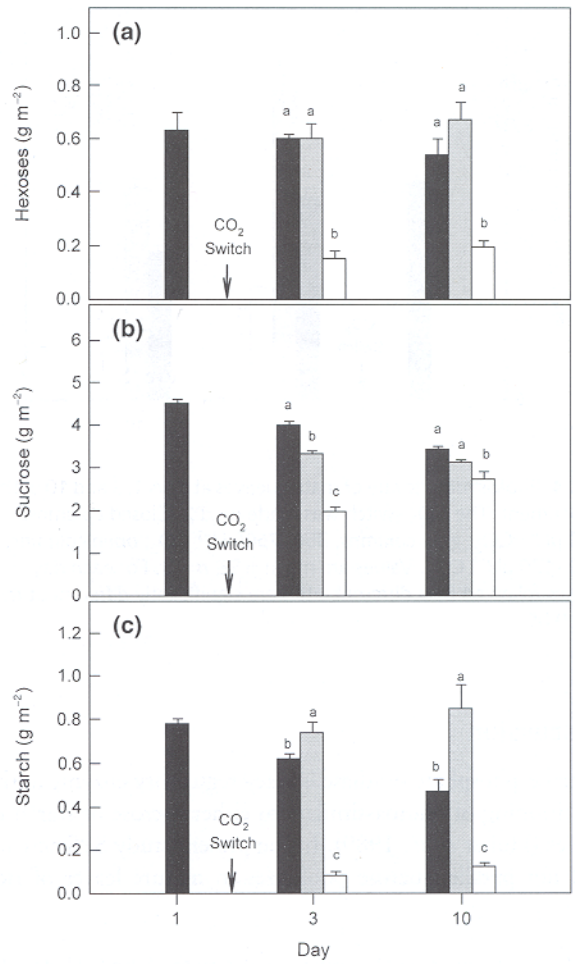


**Fig. 3** Effect of growth [$CO_2$] change on mature leaf soluble carbohydrates: hexoses (glucose and fructose) (a), sucrose (b) and starch (c) at days 1, 3 and 10 for rice switched to 700 and 175 μl $l^{-1}$ $CO_2$ and plants kept at ambient $CO_2$. The $CO_2$ switch was made on d 2. Closed columns, 350 μl $l^{-1}$ $CO_2$; gray columns, 350–750 μl $l^{-1}$ $CO_2$; open columns, 350–175 μl $l^{-1}$ $CO_2$. Values are mean ± SE, $n$ = 6–9. For each day, values followed by a different letter are significantly different at the $P$ = 0.05.

controls. Photosynthesis of leaf number seven on the main culm tended to decline across all treatments as it aged (Fig. 4).

At day 11, 9 d after switching growth [$CO_2$], canopy net photosynthesis (Pn) was 31% greater for high $CO_2$-switched rice and 36% less in low-switched plants than those kept at ambient $CO_2$ (Table 1). Canopy net dark respiration (data not shown) was similar between ambient and low $CO_2$-switched rice but was 38% greater in high $CO_2$-switched plants. Despite the considerably greater photosynthesis of high $CO_2$-switched plants only the mass of stem and sheath was significantly different ($P$ = 0.05) from that of plants kept at ambient $CO_2$ (Table 1). Switching to low $CO_2$ caused a 25% reduction in leaf area compared with ambient $CO_2$ plants 9 d after the switch (Table 1).

The stem and leaf sheath are primary sinks for photosynthetic assimilate in vegetatively growing rice. Whole plants separated from their roots were collected at days 1 and 11 to analyse the partitioning of total nonstructural carbohydrate (TNC) between leaf blades and stem and sheath tissue. By day 11 (9 d after the switch), the total amount of TNC in ambient $CO_2$ plants changed little, whereas high $CO_2$-switched rice increased 46% and low-switched plants deceased 23% (Table 2). Only the high $CO_2$-switched plants showed an increase of TNC in leaf blades by day 11, whereas all three treatments had increased amounts in stem and sheath tissue during the experiment (Table 2). However, the TNC content in stems and sheaths of high-switched plants nearly doubled and was significantly higher ($P$ = 0.05) than that in low-switched and ambient $CO_2$ plants. This indicates that for high $CO_2$-switched plants, either far more photoassimilate was transferred into stems and sheaths, or less was translocated out. Sucrose pool sizes in leaf blades and stems and sheaths paralleled the changes in TNC across $CO_2$ treatments at day 11 (Table 2). The sucrose content in stems and sheaths of high-switched plants more than tripled during the experiment and made up 73% of the TNC recovered. By contrast, at day 1, sucrose made up only 43% of the TNC. Both low-switched and ambient $CO_2$ rice showed an increase of sucrose in stems and sheaths but much less than that of high $CO_2$-switched plants.

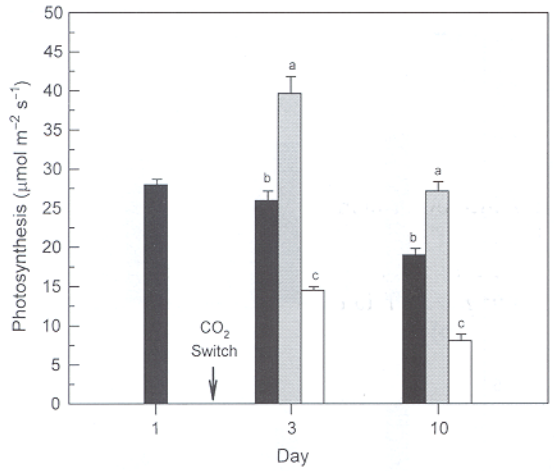


**Fig. 4** Photosynthetic rate of mature leaves at days 1, 3 and 10 of the experiment. The $CO_2$ switch was made on d 2. Closed columns, 350 μl $l^{-1}$ $CO_2$; gray columns, 350–750 μl $l^{-1}$ $CO_2$; open columns, 350–175 μl $l^{-1}$ $CO_2$. Values are mean ± SE, $n$ = 3. For each day, values followed by a different letter are significantly different at the $P$ = 0.05.

## Discussion

Sucrose-phosphate synthase is a key regulatory enzyme in the partitioning of photoassimilate to either sucrose or starch in leaves (Huber *et al.*, 1989). In the present study SPS activity did not parallel sucrose pool sizes in mature leaves of rice changed from ambient to high and low [$CO_2$]. Despite a dramatic increase in photosynthesis and greater SPS activity in mature leaves within 24 h of changing to high $CO_2$, leaf sucrose content declined and hexoses did not change. Furthermore, soluble invertase activity decreased. Although starch synthesis initially increased in high $CO_2$-switched leaves (Fig. 2b, day 3), starch content was several-fold lower than sucrose throughout the study, indicating that it was not a major factor in regulating assimilate partitioning. These findings strongly indicate that for high $CO_2$-switched plants, newly formed photosynthate was being rapidly exported from mature leaves to vegetative sinks. This is consistent with the large increase of TNC in stem and sheath tissues during the experiment, which was almost entirely due to sucrose (Table 2). Daily export rates of assimilated carbon from mature leaves of rice have been shown to increase in response to growth under elevated [$CO_2$] (Rowland-Bamford *et al.*, 1990). However, in the present study, suppressed transport from stem and sheath tissue could have resulted in the accumulation of TNC, although this is less likely.

Conversely, within 1 d of switching to low $CO_2$, $V_{lim}$ SPS activity and activation in mature leaves decreased, and this was correlated with declines in photosynthesis and soluble carbohydrate pool sizes (Figs 1b,c, 3a,b, and 4). Even by 8 d after the switch (day 10 of the experiment), SPS activity of both low and high $CO_2$-switched rice leaves was unrelated to sucrose pool sizes. The findings indicate that the regulation of sucrose synthesis was affected by feedforward rather than feedback processes following the changes in growth [$CO_2$].

**Table 1** Canopy net photosynthesis and growth of rice 1 d before (day 1) and 9 d after (day 11) changing growth [$CO_2$] to 700 and 175 μl $l^{-1}$. Plants at 350 μl $l^{-1}$ at 9 d after the switch were those maintained at ambient $CO_2$. Values are means ± SE, $n$ = 7–13 for photosynthesis and $n$ = 9 for growth parameters. SLW is specific leaf weight and LAI is leaf area Index ($m^2$ $m^{-2}$ land area)

| Growth $CO_2$ (ml $l^{-1}$) | Time relative to switch | Canopy net photosynthesis (mmol $CO_2$ $m^{-2}$ $s^{-1}$) | Stem and sheath d. wt (g per plant) | Leaf area ($dm^2$ per plant) | Leaf d. wt (g per plant) | SLW (g d. wt $dm^{-2}$) | LAI |
|---|---|---|---|---|---|---|---|
| 350 | 1 d before | 33.8 ± 0.5 | 0.43 ± 0.1 | 2.03 ± 0.2 | 0.50 ± 0.1 | 0.25 | 5.08 |
| 350 | 9 d after | 36.6 ± 0.9b | 0.87 ± 0.1b | 3.11 ± 0.1a | 0.89 ± 0.1ab | 0.29 | 7.50 |
| 350–700 | 9 d after | 48.1 ± 1.0a | 1.22 ± 0.1a | 3.42 ± 0.4a | 1.10 ± 0.1a | 0.32 | 8.24 |
| 350–175 | 9 d after | 23.6 ± 0.3c | 0.67 ± 0.1b | 2.33 ± 0.2b | 0.77 ± 0.1b | 0.33 | 5.62 |

Values within columns followed by a different letter are significantly different at $P$ = 0.05.

**Table 2** The partitioning of total non-structural carbohydrate (TNC) and Sucrose in leaf blades and stem and sheaths of rice 1 d before (day 1) and 9 d after (day 11) changing growth [$CO_2$] to 700 μl $l^{-1}$ and 175 μl $l^{-1}$. Plants at 350 μl $l^{-1}$ 9 d after the switch were those kept at ambient $CO_2$. The $CO_2$ switch was made on day 2. Values are mean ± SE, $n$ = 6–9.

| Growth $CO_2$ (μl $l^{-1}$) | Time relative to switch | TNC Leaf blade (mg $g^{-1}$ d. wt) | Stem and sheath) (mg $g^{-1}$ d. wt) | Total above-ground (mg $g^{-1}$ d. wt) | Sucrose Leaf blade (mg $g^{-1}$ d. wt) | Stem and sheath (mg $g^{-1}$ d. wt) |
|---|---|---|---|---|---|---|
| 350 | 1 d before | 49.9 ± 3 | 36.7 ± 4 | 86.6 ± 7 | 26.4 ± 2 | 15.9 ± 3 |
| 350 | 9 d after | 32.9 ± 2b | 48.4 ± 7b | 81.3 ± 9b | 17.3 ± 3b | 26.4 ± 4b |
| 350–700 | 9 d after | 57.2 ± 5a | 68.7 ± 9a | 125.9 ± 14a | 35.1 ± 5a | 50.3 ± 6a |
| 350–175 | 9 d after | 26.2 ± 2c | 40.7 ± 7b | 66.9 ± 9c | 14.5 ± 3b | 25.7 ± 4b |

Values within columns followed by a different letter are significantly different at $P$ = 0.05.

How then might have SPS activity been affected by feed-forward processes? The regulation of SPS varies with species (Huber *et al.*, 1989). Its activity is subject to coarse control via synthesis of enzyme protein and by covalent modification via phosphorylation/dephosphorylation (Huber & Huber, 1996), and by fine control via its allosteric effectors glucose-6-P (activator) and Pi (inhibitor) (Stitt *et al.*, 1988). In the present study, differences measured *in vitro* were not due to allosteric control since leaf extracts were desalted before assaying and substrate and effector concentrations were kept constant.

Changes in SPS activity, especially within 24 h after the $CO_2$-switch, were likely to be due to covalent modification of the enzyme. We postulate that modified SPS activity resulted from changes in the flux of triose-P (dihydroxy acetone phosphate (DHAP) glyceral dehyde phosphate (GAP) and phosphoglycerate (PGA) ) and Pi between the chloroplast and cytosol caused by changes in photosynthesis coupled to sink demand. Phosphorylation of SPS causes deactivation, while dephosphorylation reactivates the enzyme, which in part is regulated by cytosolic [Pi] (Huber & Huber, 1992). Regulation of SPS in this manner can occur rapidly (Huber *et al.*, 1994) and is highly dependent on photosynthate flux and demand for sucrose (Stitt *et al.*, 1988).

Photosynthesis was significantly reduced in mature leaves of plants switched to low $CO_2$ (Fig. 4). It is likely that low $CO_2$-switched leaves experienced greater cytosolic [Pi] than their ambient counterparts owing to reduced triose-P/Pi flux between chloroplasts and cytoplasm. Therefore, SPS phosphorylation may have been greater (i.e. inactive state *in vivo*) and would explain why $V_{lim}$ activity and activation of the enzyme were lower than that of ambient $CO_2$ leaves despite no difference in $V_{max}$ (Fig. 1). Gerhardt *et al.* (1987) using *Spinacia oleracea* L. leaves has shown evidence suggesting cytosolic [Pi] increases in response to decreased photosynthesis under limiting [$CO_2$]. It should be noted that in the present study, coregulation of sucrose synthesis via fructose-1,6-bisphosphatase cannot be ruled out since its activity was not measured.

By contrast, within 24 h of switching to high $CO_2$, both SPS $V_{max}$ and $V_{lim}$ activities increased (Fig. 1a,b) parallel with photosynthesis (Fig. 4). This is likely to have resulted from both increased hexose-P synthesis and triose-P cycling keeping cytosolic [Pi] relatively low. These two processes can act in concert to increase SPS activity (Huber & Huber, 1992). Stitt *et al.* (1983) has shown that the flux of triose-P and the level of hexose-P in the cytosol increase with photosynthesis. Despite the lack of change in the hexose pool and a decrease in sucrose for high $CO_2$-switched leaves (Fig. 3), whole-plant data indicated that assimilate was being exported to sink tissues (Table 2). Therefore, it is reasonable to assume that hexose-P synthesis and triose-P flux in high-switched leaves were greater than that of ambient $CO_2$ controls. Increased sucrose synthesis with little change in metabolite levels has been noted elsewhere (Neuhaus *et al.*, 1990). Increased SPS activity owing to greater enzyme protein synthesis cannot be ruled out. However, it is less likely as changes in SPS gene expression and protein synthesis can take several days following modifications in photosynthesis (Klein *et al.*, 1993).

Acid-soluble invertase activity is often negatively correlated to sucrose content in leaves (Huber, 1989; Goldschmidt & Huber, 1992). This was not the case when source–sink balance was modified by changing growth [$CO_2$]. At day 3, invertase activity in mature leaves of both $CO_2$-switched treatments paralleled the decline in sucrose content (Figs. 2a and 3b). This finding, in addition to the low starch contents that were found, further indicates that assimilate partitioned to sucrose was exported rather than accumulated in mature leaves.

The significance of increasing partitioning of assimilate to sucrose under high photosynthetic rates may be to avoid, at least in the short-term, carbohydrate feedback inhibition of photosynthesis. Galtier *et al.* (1995) using *Lycopersicon esculentum* L. and Signora *et al.* (1998) using *Arabidopsis*, have shown that transformed plants over-expressing SPS had greater photosynthetic rates than untransformed controls when both were grown under high $CO_2$. Also, *Arabidopsis* leaves over-expressing SPS accumulated less soluble sugars than untransformed controls when grown for 10 wk at 700 μl $l^{-1}$ [$CO_2$] (Signora *et al.*, 1998). In our study, 11 d after switching [$CO_2$], canopy Pn was compared across treatments at common [$CO_2$]s (data not shown) with no indication of carbohydrate feedback inhibition of photosynthesis in plants switched to high $CO_2$.

In conclusion we have shown that changing growth [$CO_2$] late in vegetative development of rice led to rapid alterations in sucrose metabolism that were not associated with sucrose pool sizes. Switching to high $CO_2$ significantly stimulated photosynthesis and triggered a rapid increase of SPS activity in mature leaves. Hence, photoassimilate was partitioned to sucrose and was exported to vegetative sinks, thus preventing an accumulation of soluble carbohydrates. Conversely, the change to low $CO_2$ reduced photosynthesis and led to decreased activation of SPS. These data indicate that adjustments in the regulation of SPS immediately following source/sink modification by changes in growth [$CO_2$] are linked to feed-forward processes.

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